

TITLE \_\_\_\_\_

Project No. 1713  
Book No. 19952

Exhibit K, pg. 1 of 33

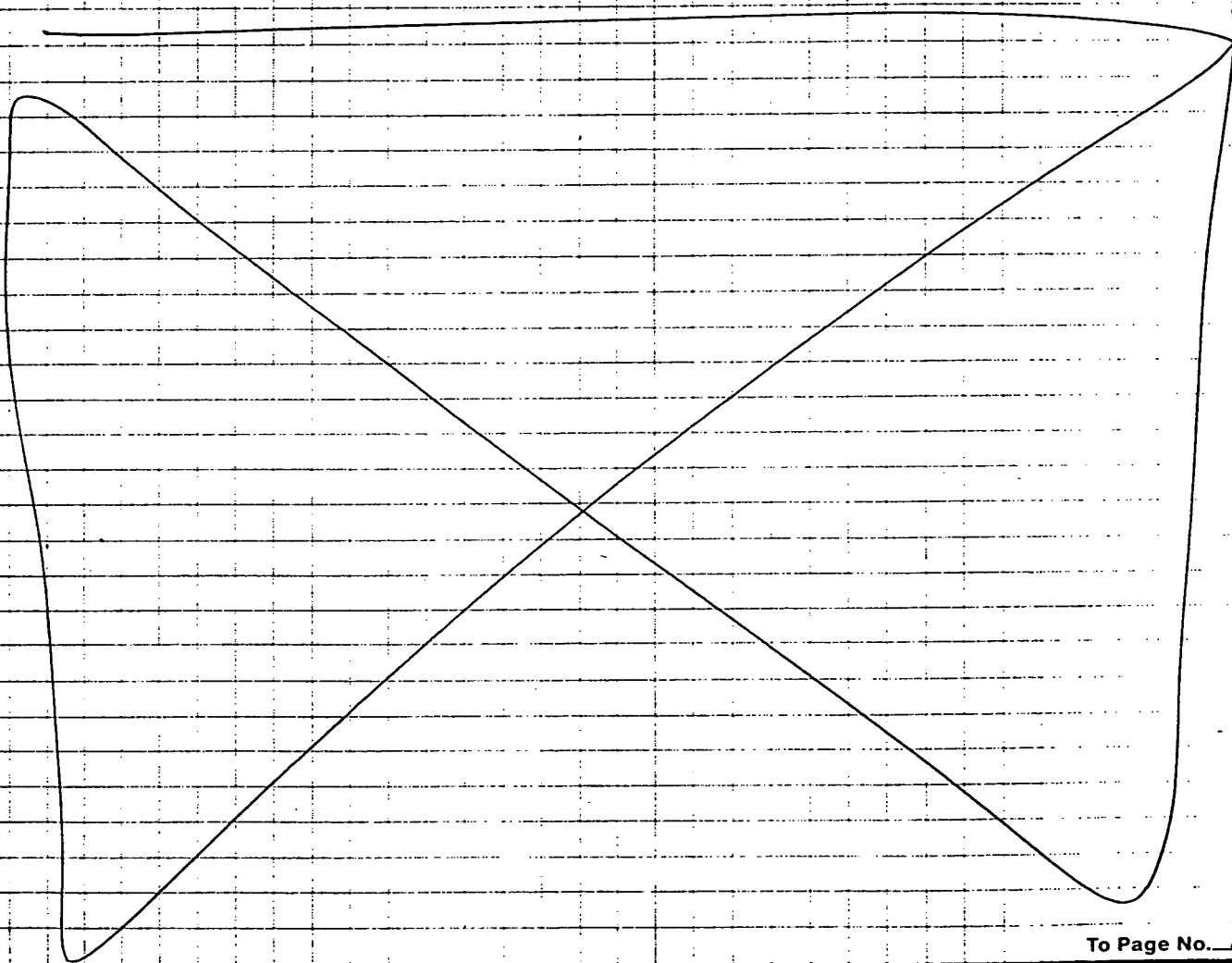
From Page No. 6

Checked spinners + plate

Split spinners

Got O.K. (opossum kidney) cell line from B. Matthews

Getting ready to try FACS screening of cell lines  
using TRb IgG fusion to stain.



To Page No. 8

Witnessed & Understood by me,

Date

Invented by

Date MON

Recorded by

Will Bacon

10/25/93

8

From Page No. 7

Split CHO FUS 9+11 spinners  
Split CHO FUS 9+11 plates

Split D.K. cells & scaled up for FACS.

RS'd 2° + 3° Antibodies from Jackson for FACS.

To Page No. 9

Witnessed & Understood by me,

Date

Invented by

Date WED

Recorded by

10/27/93

|||

Proj. No. 1713  
Book No. 1995

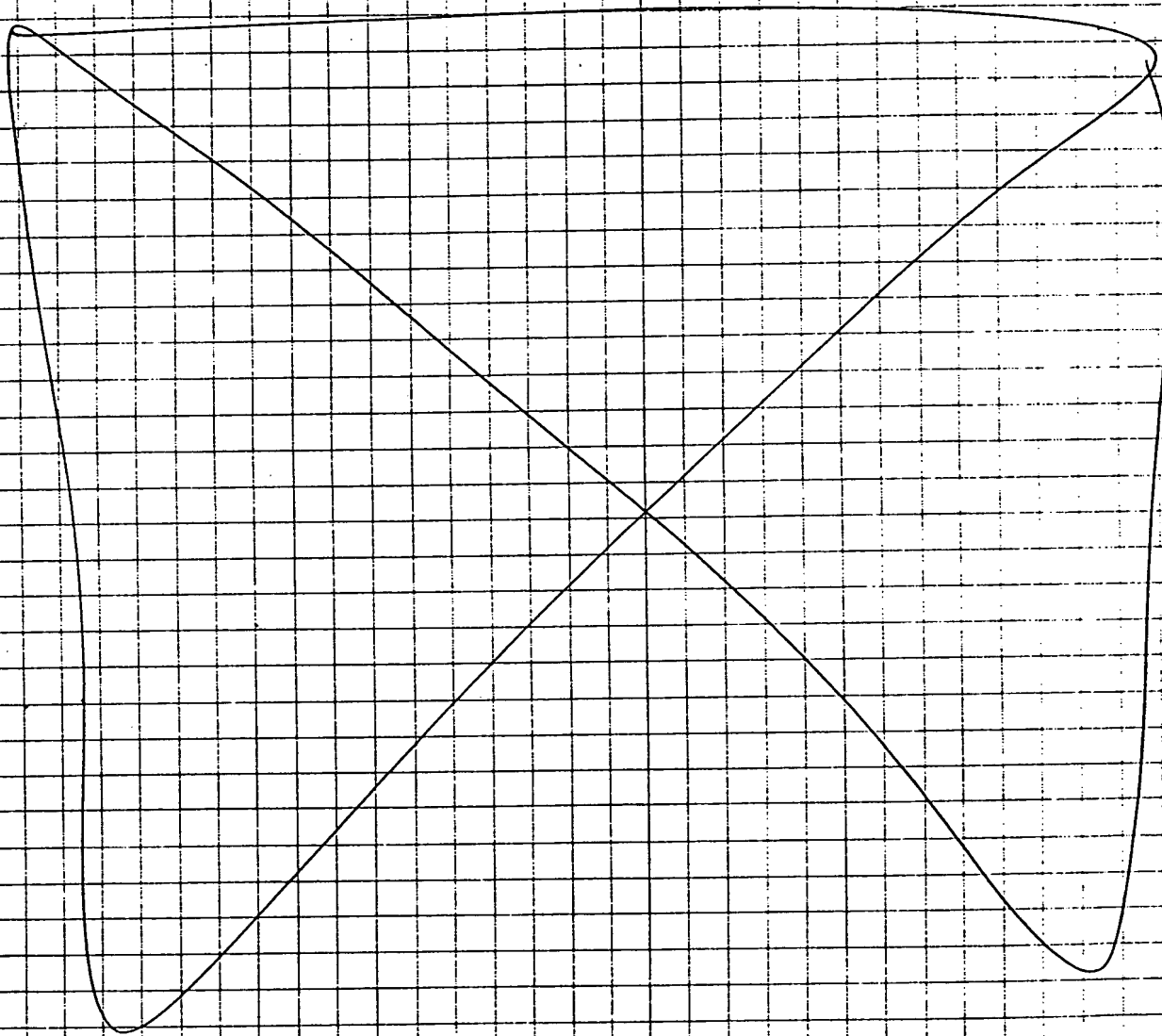
Exhibit K, pg. 3 of 33

TITLE \_\_\_\_\_

From Page No. 8

*Split spinners*

*Checked all other cells*



To Page No. 10

Witnessed & Understood by me, \_\_\_\_\_

Date \_\_\_\_\_

Invented by \_\_\_\_\_

Date FR1

Recorded by \_\_\_\_\_

*Will Bacon*

10/29/93

Projec. B. 1713  
Book No. 19952 TITLE \_\_\_\_\_

10

From Page No. 9*Split all cells & spinners*To Page No. 11

Witnessed &amp; Understood by me,

Date

Invented by

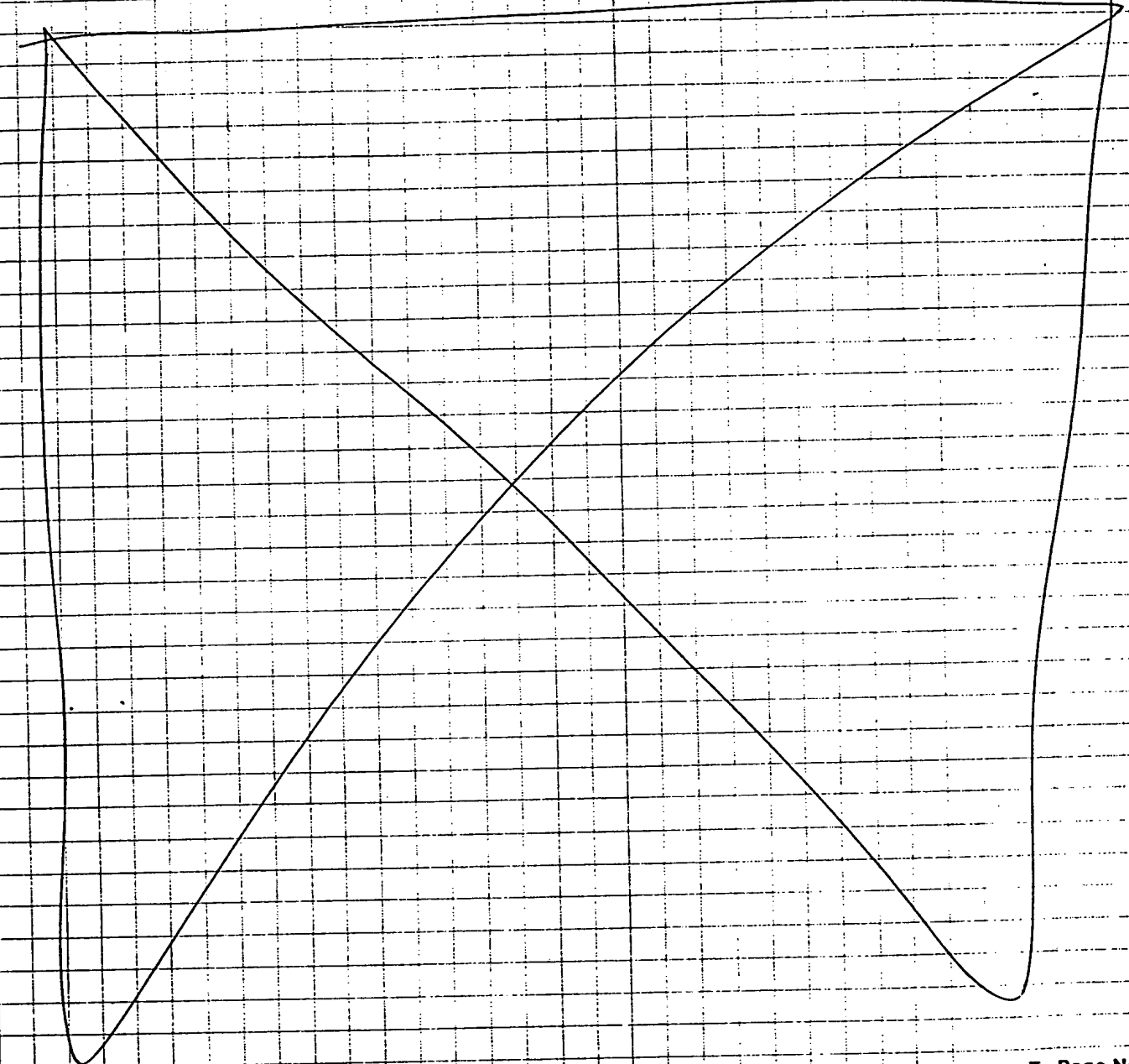
Date MON

Recorded by

WMB  
11/1/93

Proj. No. 1713  
Book No. 19952

TITLE \_\_\_\_\_

From Page No. 10*split all spinners & cells*To Page No. 12

Witnessed &amp; Understood by me,

Date

Invented by

Date FR1

Recorded by

*W. H. Bacon*11/5/93

Project No. 1713  
Book No. 19952 TITLE \_\_\_\_\_

12

From Page No. 11

Split spinners & plates

Trained 20 full length HPTK6 / CHO 1s.

Have polyclonal antisera so I can screen

these for expression now.

To Page No. 13

Witnessed &amp; Understood by me,

Date

Invented by

Date WED

Recorded by

W. A. Baron  
11/10/93

TITLE

From Page No. 12

Changed media on all full length Thaws.  
Need to make sure that there is no GHT around

Got Glioma 5120 from J. Lee  
Split

To Page No. 14

Witnessed & Understood by me,

Date

Invented by

Recorded by

Date THURS

11/11/93

Project No. 1713  
Book No. 19952 TITLE \_\_\_\_\_

14

From Page No. 13

Split all spinners

Split all CHO cells

FUS9, LI

\* 20 full lengths

Split O.K. \* 5120 lines

To Page No. 15

Witnessed &amp; Understood by me,

Date

Invented by

Recorded by

Date AT11/13/93

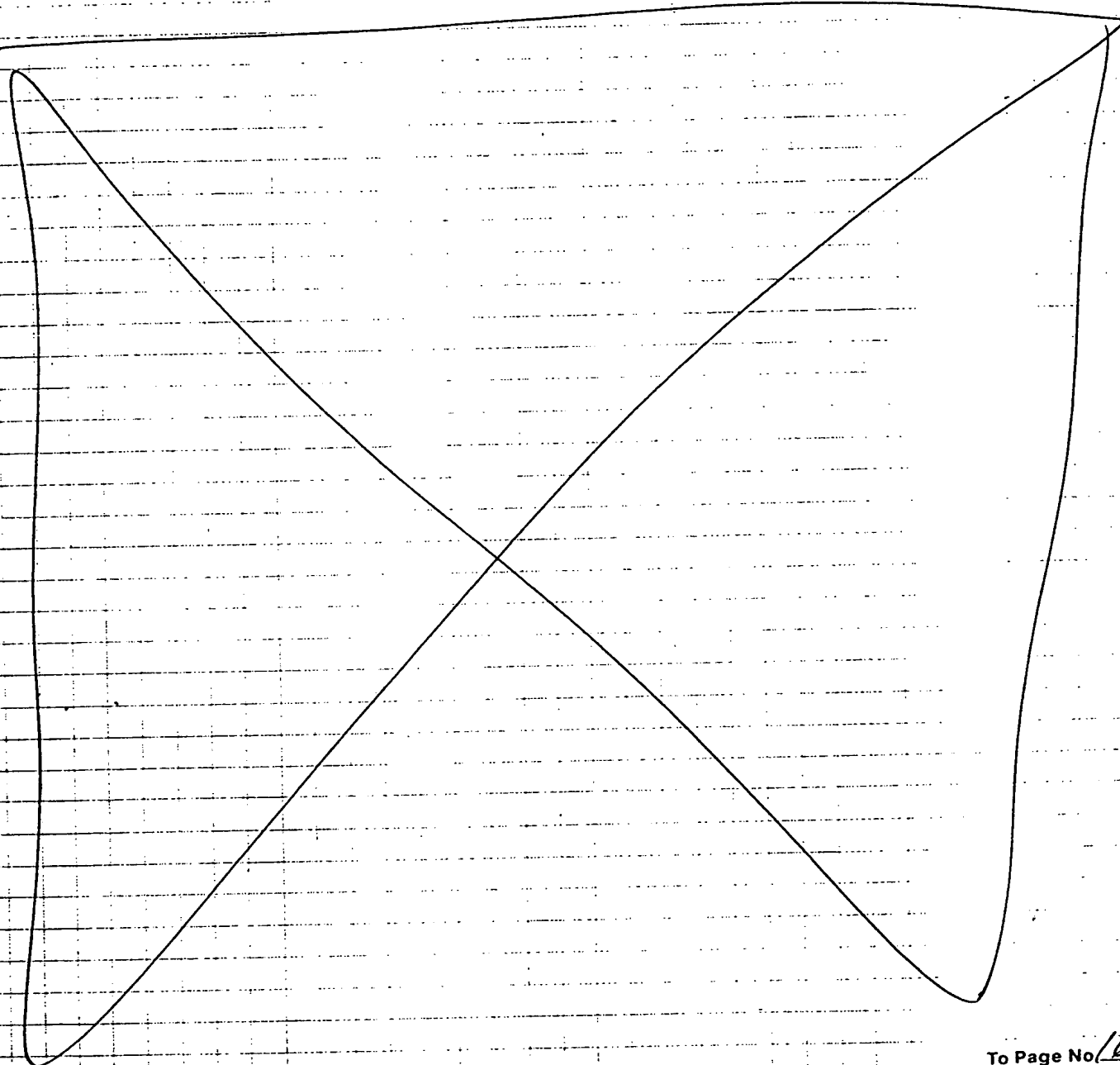


Project No. 1713  
Book No. 19952

TITLE \_\_\_\_\_

From Page No. 14

*Split all cells & spinners*



To Page No. 16

Witnessed & Understood by me, \_\_\_\_\_

Date \_\_\_\_\_

Invented by \_\_\_\_\_

Recorded by \_\_\_\_\_

Date \_\_\_\_\_

WED

11/17/93

Project No. 1713  
Book No. 19952 TITLE

16

From Page No. 15

Split all FUS plates  
split all spinners & scaled up for  
more PS04 runs

Split D.K., 6120 & 487 cell lines  
for FACS on 11/23.

split CHO TK6 full lengths & seeded  
6 well dishes at 500,000 each clone.

To Page No. 17

Witnessed &amp; Understood by me,

Date

Invented by

Recorded by

Date Mon

11/22/93

TITLE \_\_\_\_\_

From Page No. 16

FACS analysis for TK6 Ligand:

Method:

Harvest cells of interest using EDTA + for scraping  
Wash in PBS + 10% goat serum, 5% rabbit serum + azide  
Block in this  $\times$  for 30' on ice

Spin  
RS in 250ul Buffer w/ serum & add 1 $^{\circ}$  stain  
which is going to be FCD/ISS fusion prot  
Mix & Inc on ice 30'  
Wash 2x PBS + azide

RS in 250ul & incubate w/ 2 $^{\circ}$  Ab w/ which is  
of Fc ~~conjugated~~ conjugated to ~~streptavidin~~ biotin  
Inc 30' on ice  
Wash 2x PBS + azide

RS in 250ul PBS + azide & bind w/  
Streptavidin / Phycoerythrin  
30' on ice  
Wash 2x PBS + azide  
RS  $\rightarrow$  add PI  $\rightarrow$  Run FACS.

I harvested OK, 6120 & 487 using  
EDTA  
Counted & Aliquoted  $2 \times 10^6$  cells per tube  
(Coulter) (5 tubes each)

Followed protocol as above using  
following stains  $\rightarrow$

No. 17

To Page No. 18

Witnessed & Understood by me,

Date

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Recorded by

Date TUES

11/23/93

W. M. Falcon

From Page No. 17

44 = OK + HPTK6 (1 $\mu$ g) + 2° + SPE  
 45 = OK + HPTK6 (5 $\mu$ g) + 2° + SPE  
 46 = OK + HPTK6 (10 $\mu$ g) + 2° + SPE  
 47 = OK + CD4 (1 $\mu$ g) + 2° + SPE  
 48 = OK + 2° + SPE

49 = G120 + HPTK6 (1 $\mu$ g) + 2° + SPE  
 50 = G120 + HPTK6 (5 $\mu$ g) + 2° + SPE  
 51 = G120 + HPTK6 (10 $\mu$ g) + 2° + SPE  
 52 = G120 + CD4 + 2° + SPE  
 53 = G120 + 2° + SPE

54 = U87 + HPTK6 (1 $\mu$ g) + 2° + SPE  
 55 = U87 + HPTK6 (5 $\mu$ g) + 2° + SPE  
 56 = U87 + HPTK6 (10 $\mu$ g) + 2° + SPE  
 57 = U87 + CD4 + 2° + SPE  
 58 = U87 + 2° + SPE

Ran out of time so I fixed cells w/  
para formaldehyde & stored 4°C o/n.

CHO TK6 full lengths were too dilute to run.  
I will analyze them later.

To Page No. 19

Witnessed &amp; Understood by me,

Date

Invented by

Date TUES

Recorded by

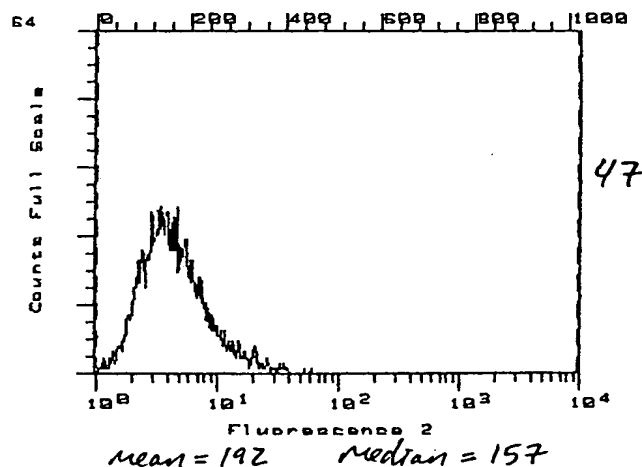
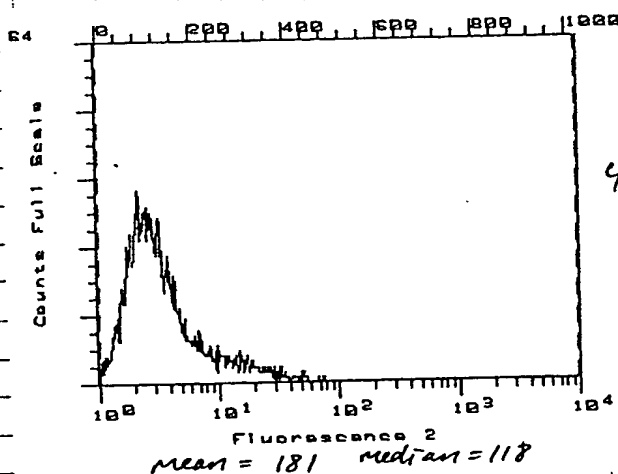
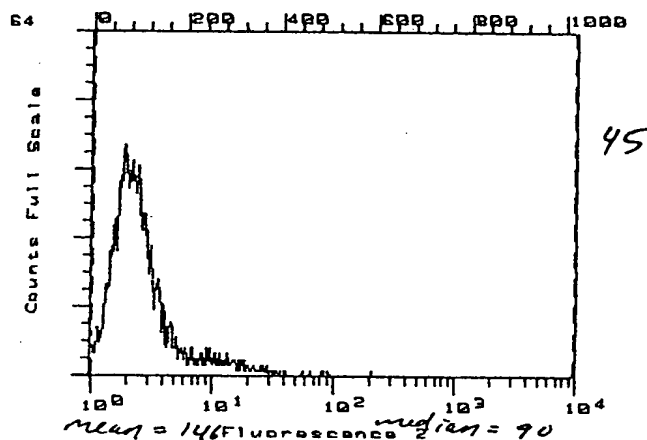
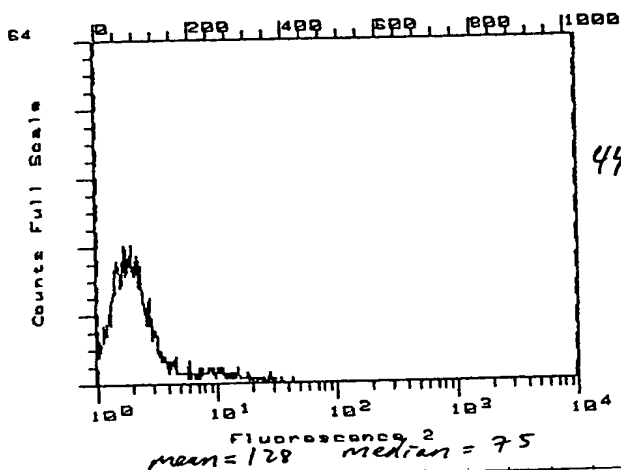
W. J. Bacon  
11/23/93

TITLE \_\_\_\_\_

From Page No. 8

Started another P504 run on both FUS 9 & FUS 11  
 Split regular spinners.  
 Split C40 full lengths 1:20  
 " C40 FUS 9 & 11 1:20

Washed Fixed cells for FACS → RAN FACS.



To Page No. 20

Witnessed & Understood by me, \_\_\_\_\_

Date \_\_\_\_\_

Invented by \_\_\_\_\_

Recorded by \_\_\_\_\_

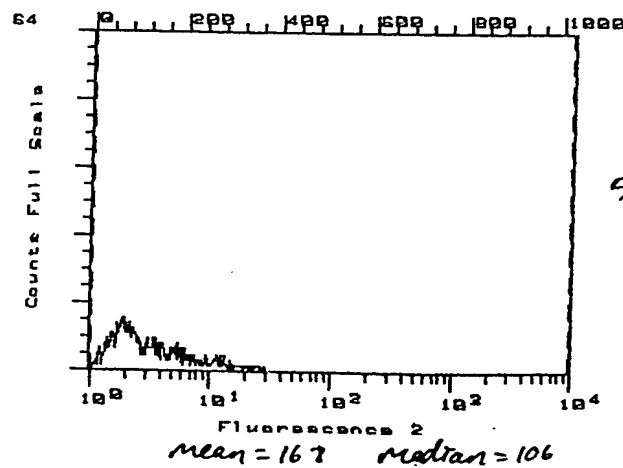
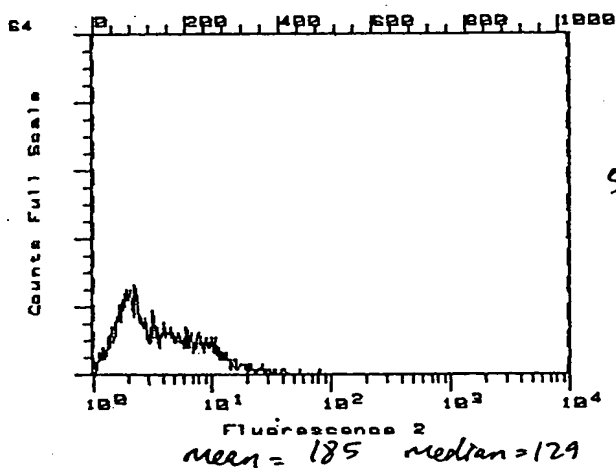
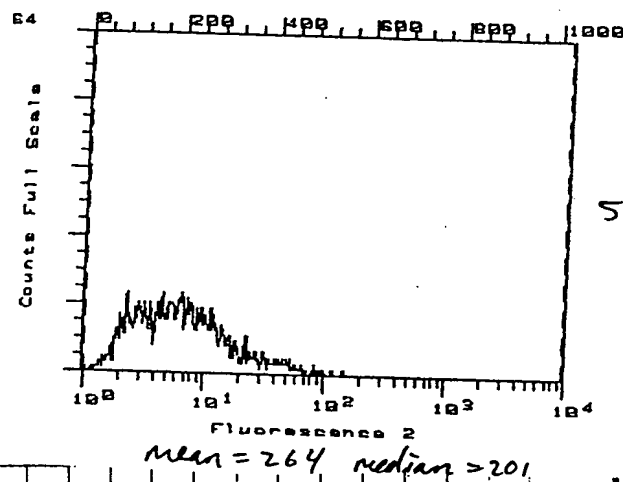
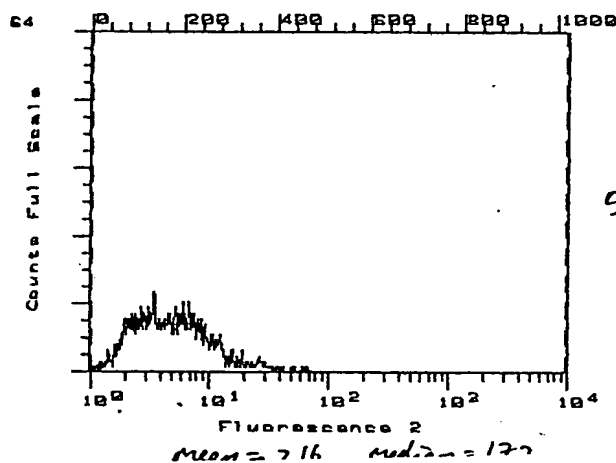
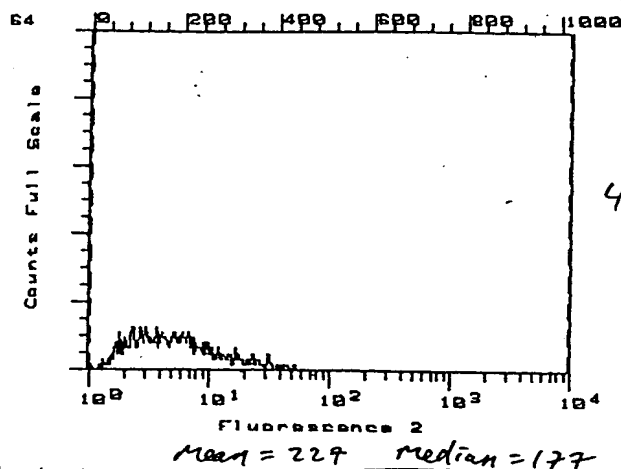
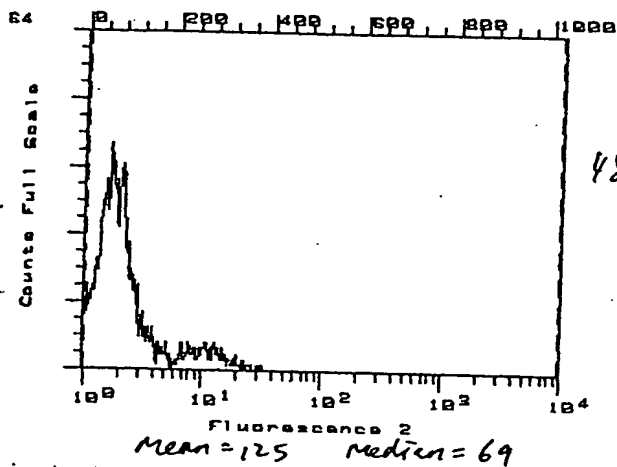
Date WED

11/24/93

19

20

From Page No. 19



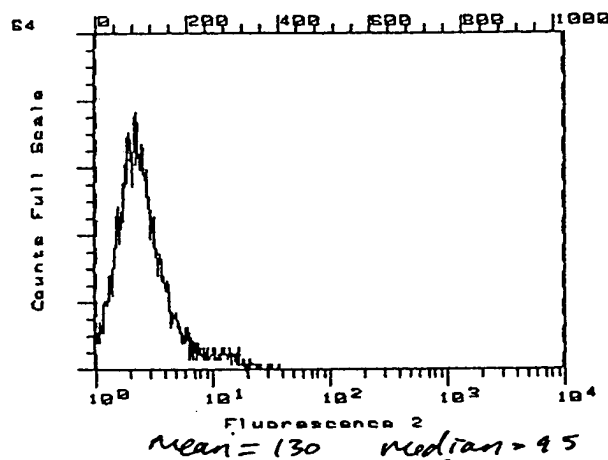
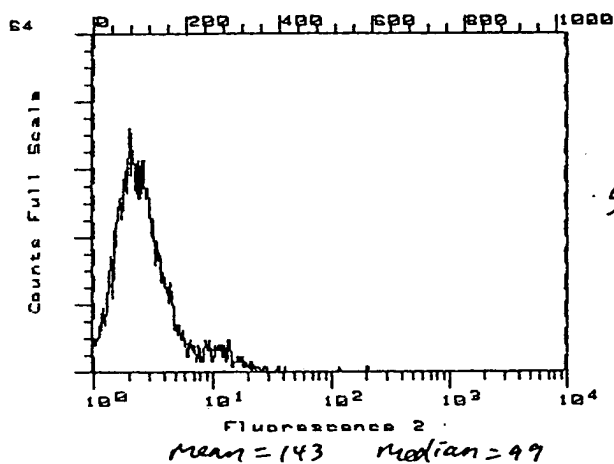
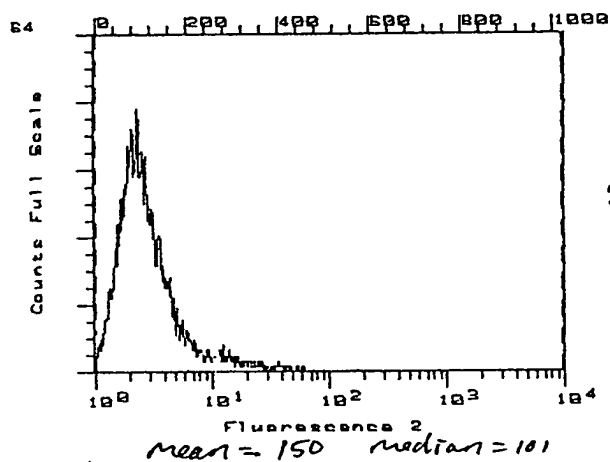
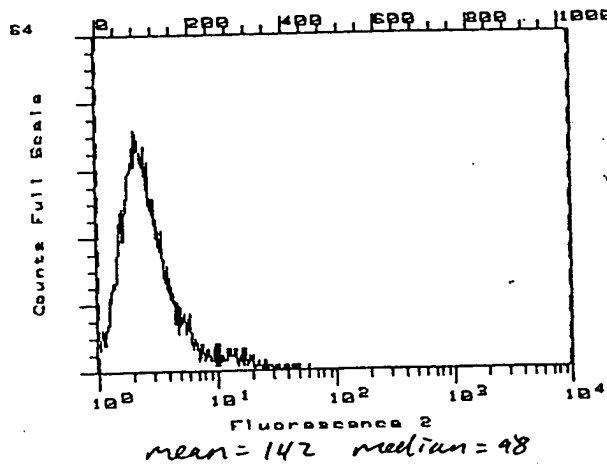
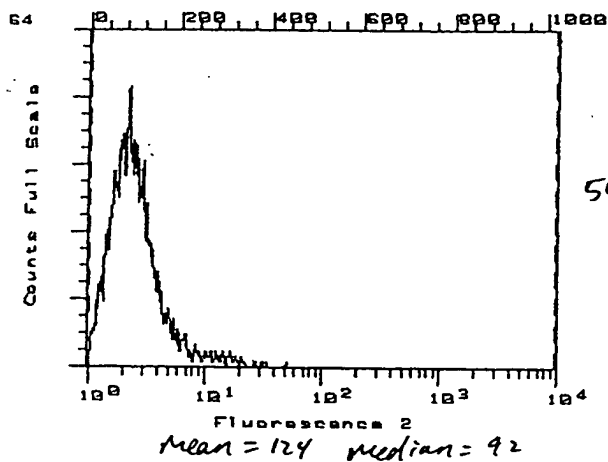
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Recorded -

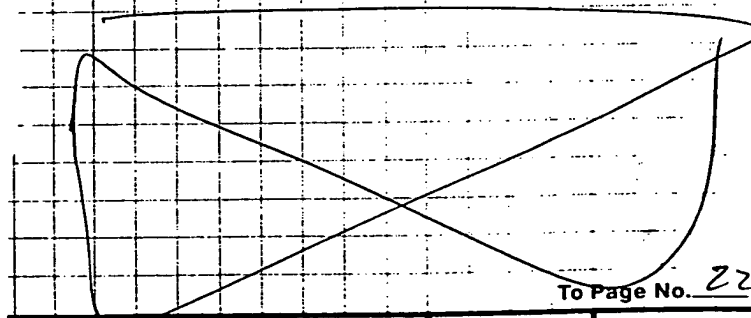
TITLE

Project No. 671  
 Box No. 1995

Exhibit K, pg. 15 of 33



No appreciable shifts



To Page No. 22

rented by

Date

Recorded by

WED  
11/24/93

22

Project No. 1713  
Book No. 19952 TITLE \_\_\_\_\_From Page No. 21split all spinners + plateschecked P504 culturesTo Page No. 23

Witnessed &amp; Understood by me,

Date

Invented by

Date MON

Recorded by

11/29/93



TITLE \_\_\_\_\_

From Page No. 22 FACS analyzed 1st 20 CHO TK6 full lengths

KEY							
Tube #				Tube #			
1)	Clone	1	Pre immune	21)	Clone	1	Immune
2)		2		22)		2	
3)		3		23)		3	
4)		4		24)		4	
5)		5		25)		5	
6)		6		26)		6	
7)		7		27)		7	
8)		8		28)		8	
9)		9		29)		9	
10)		10		30)		10	
11)		11		31)		11	
12)		12		32)		12	
13)		13		33)		13	
14)		14		34)		14	
15)		15		35)		15	
16)		16		36)		16	✓ ①
17)		17		37)		17	✓
18)		18		38)		18	
19)		19		39)		19	
20)		20		40)		20	

Tube #  
 41) CHO / DP-12 Pre-immune  
 42) CHO / DP-12 Immune

To Page No. 24

Witnessed & Understood by me, \_\_\_\_\_

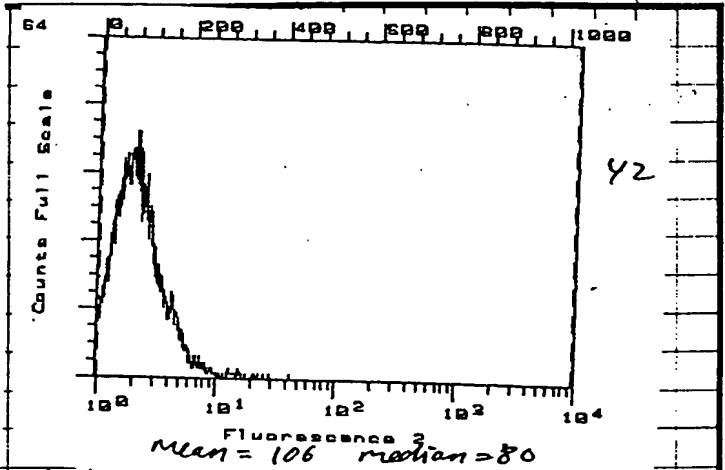
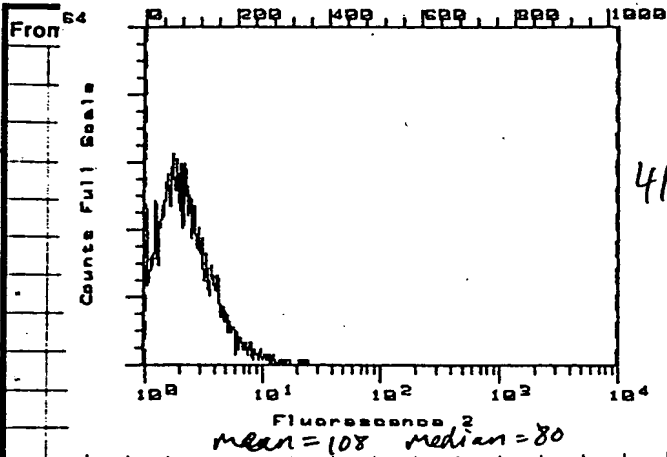
Date \_\_\_\_\_

Invented by \_\_\_\_\_

Recorded by \_\_\_\_\_

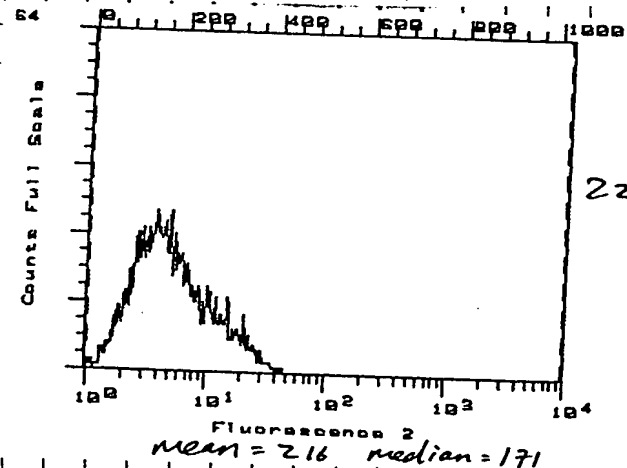
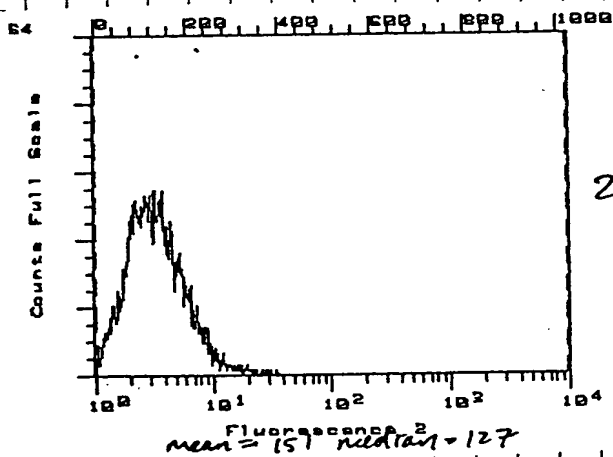
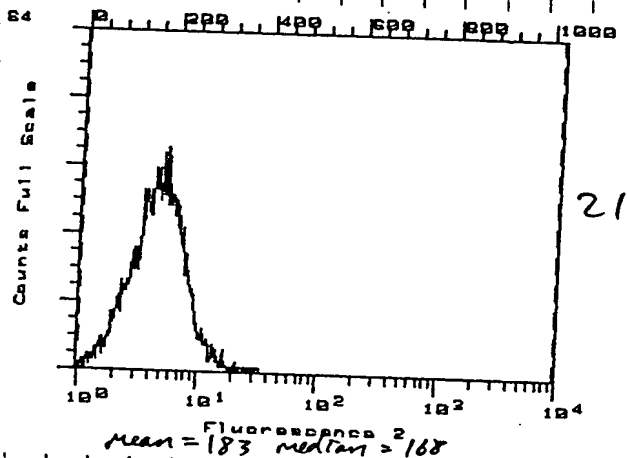
Date THURS

12/2/93



PRINTER  
PROBLEM

#1 LOST

To Page No. 25

Witnessed &amp; Understood by me,

Date

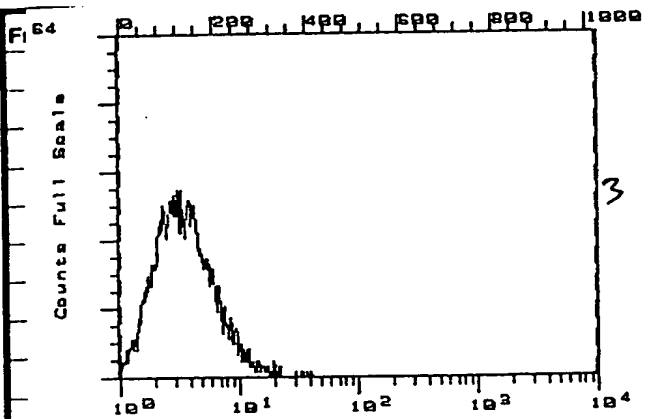
Invented by

Date THURS

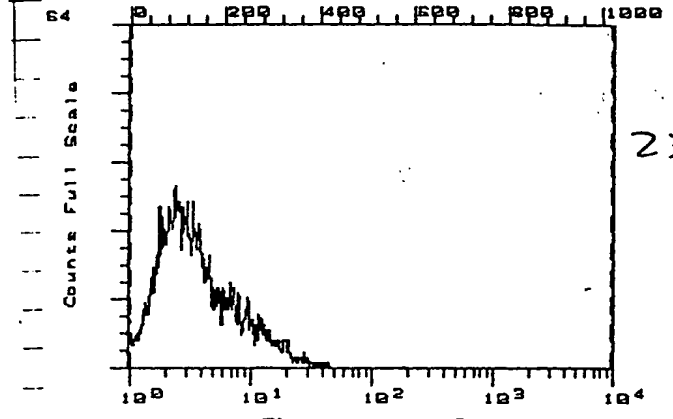
Recorded by

12/2/93

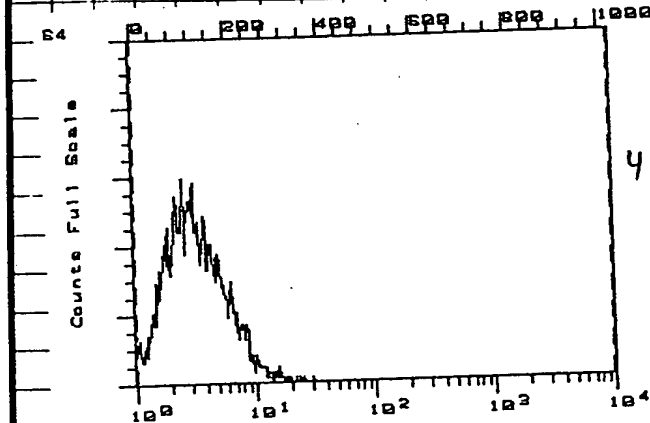
TITLE



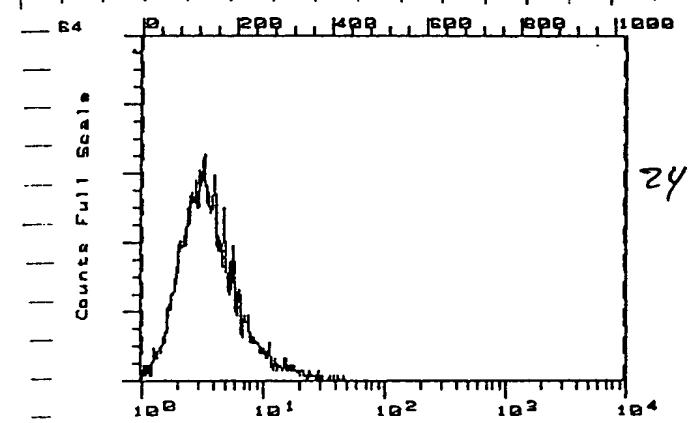
mean = 158 median = 134



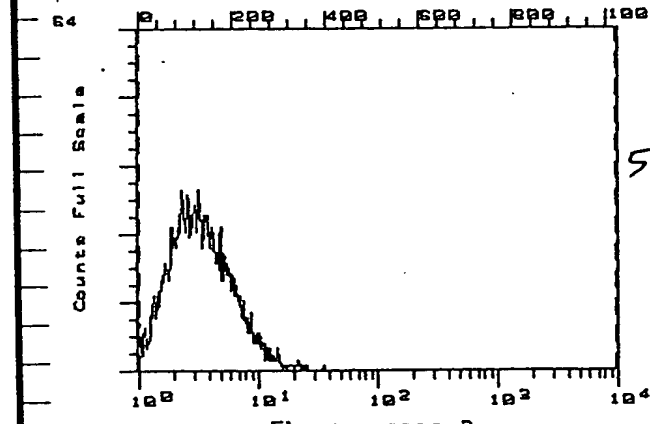
mean = 180 median = 128



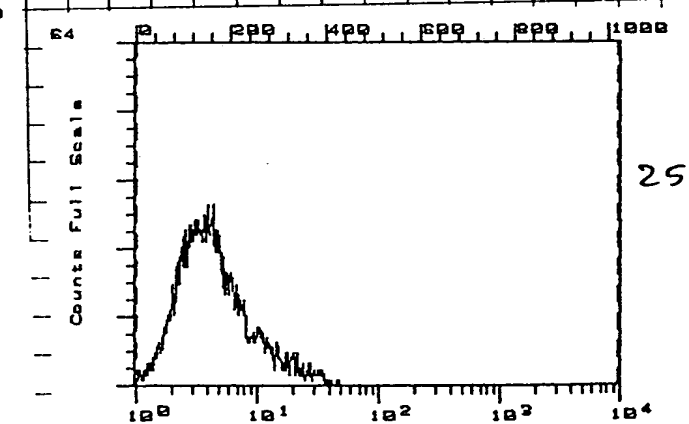
mean = 148 median = 128



mean = 166 median = 135



mean = 154 median = 128



mean = 197 median = 155

To Page No. 26

Witnessed & Understood by me,

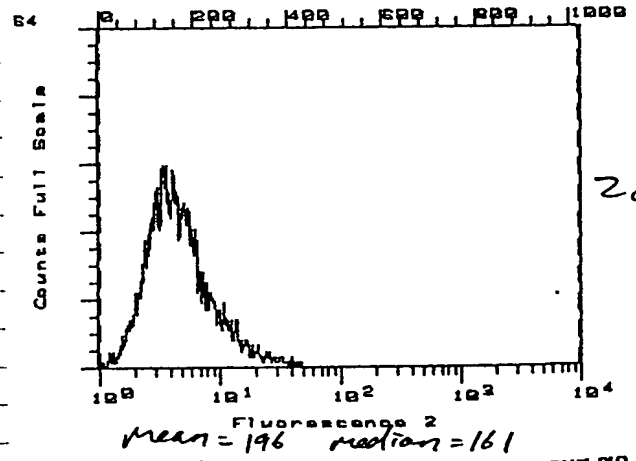
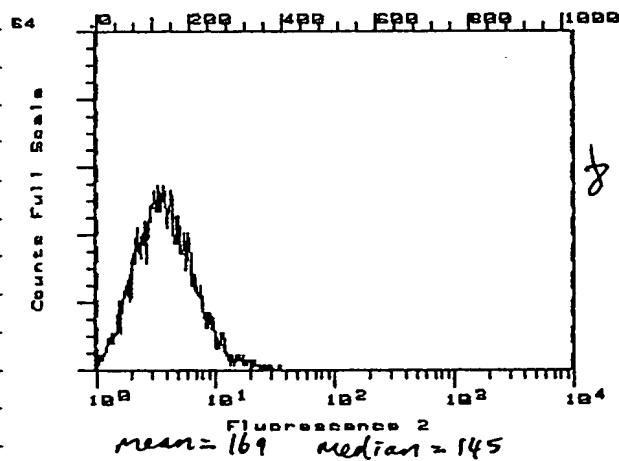
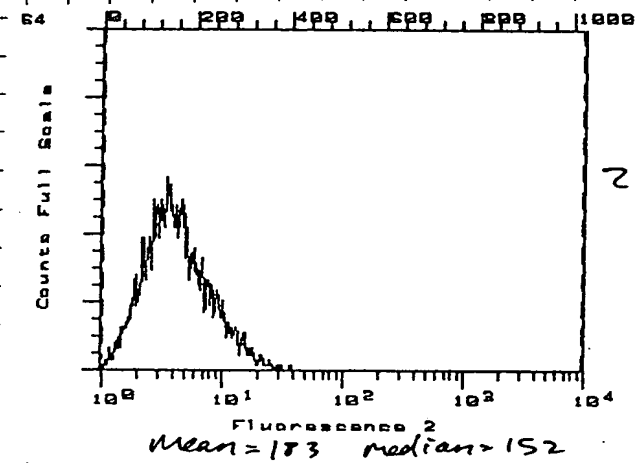
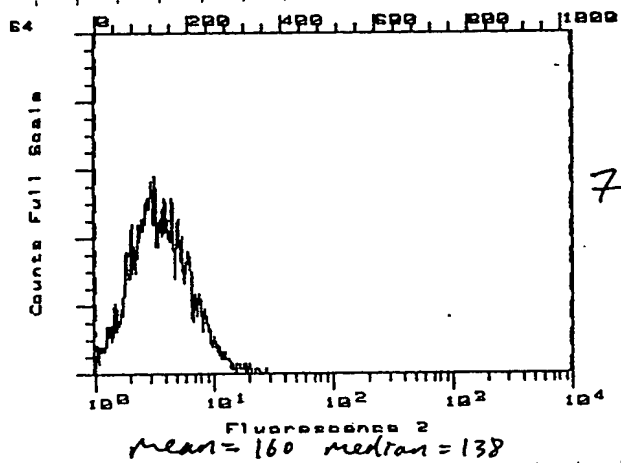
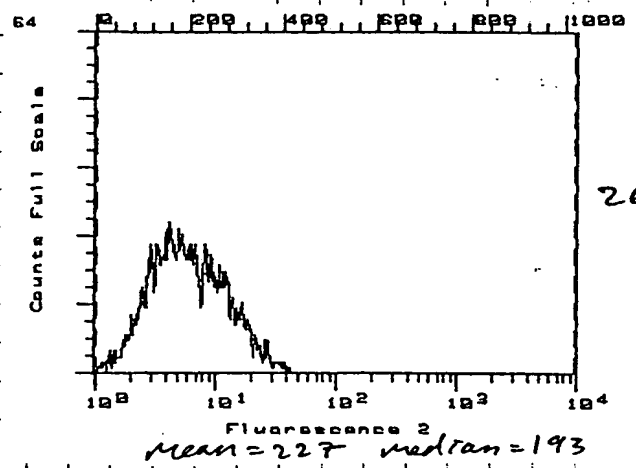
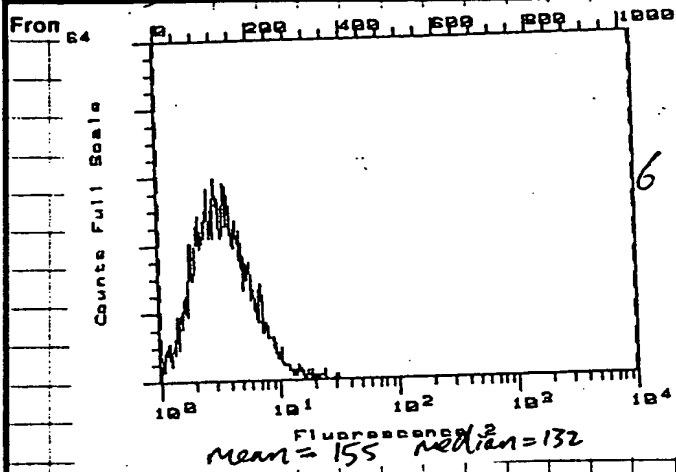
Date

Invented by

Recorded by

Date THURS

12/2/93



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Date

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Date THURS

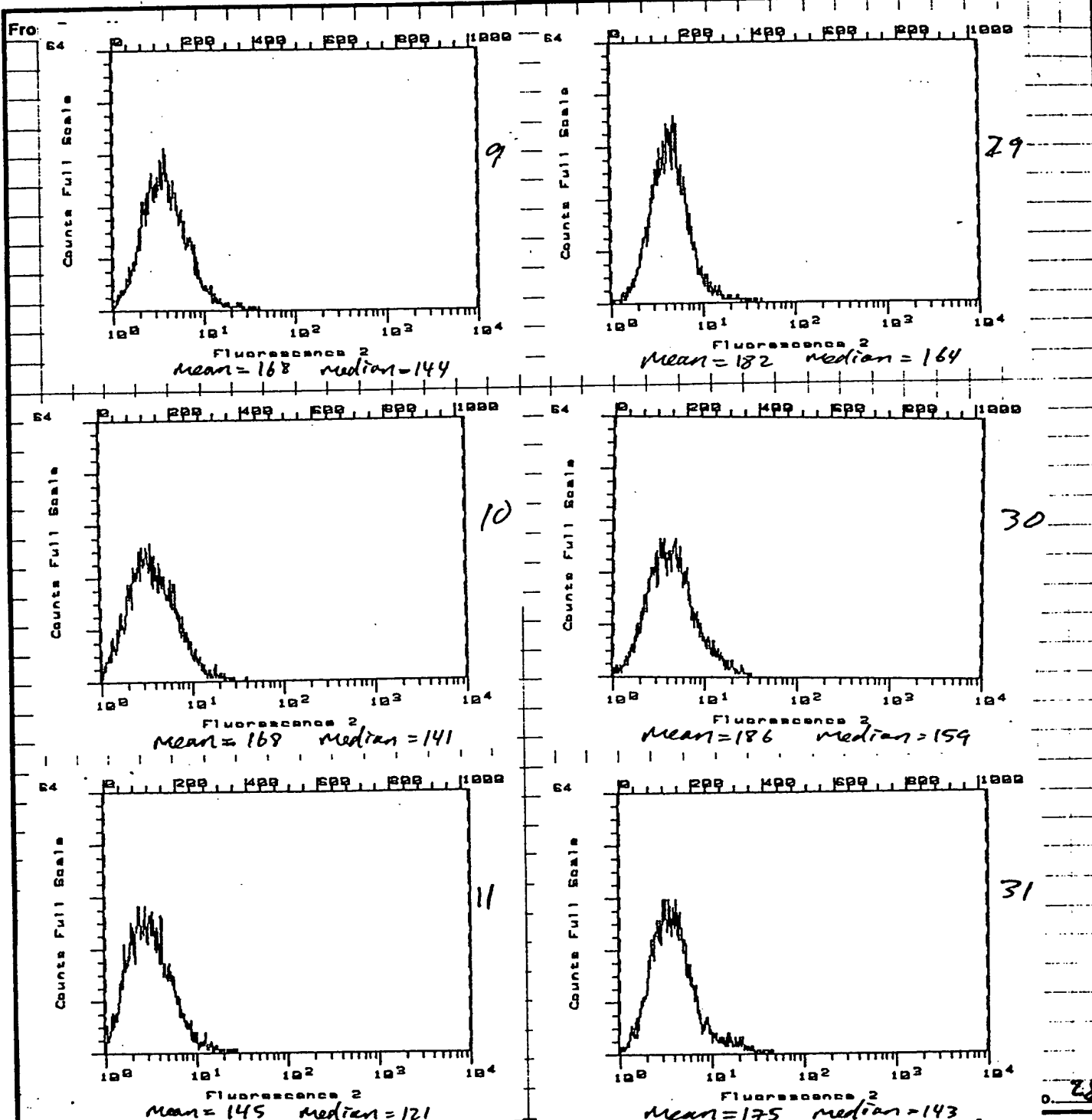
Recorded by

WILL BARON

12/2/93

Page No. 27

TITLE \_\_\_\_\_



Witnessed & Understood by me, \_\_\_\_\_

Date \_\_\_\_\_

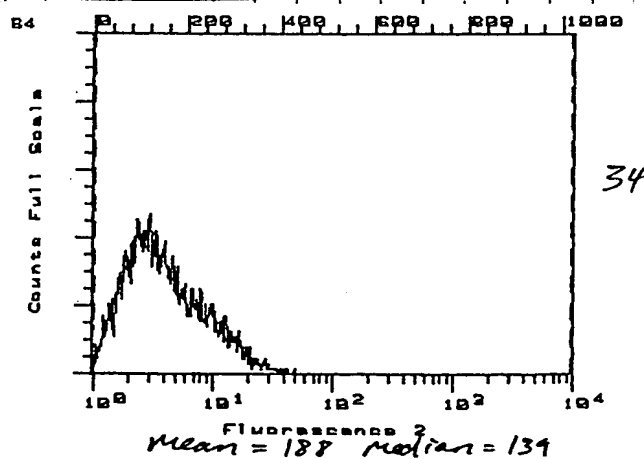
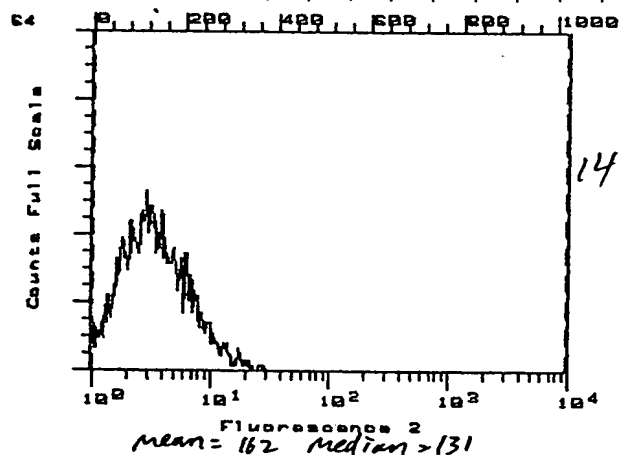
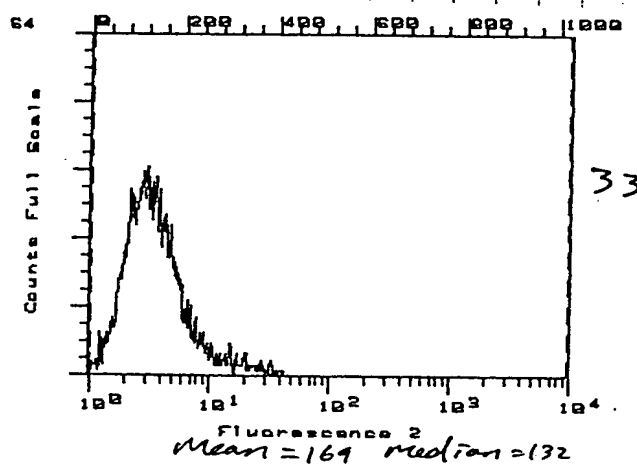
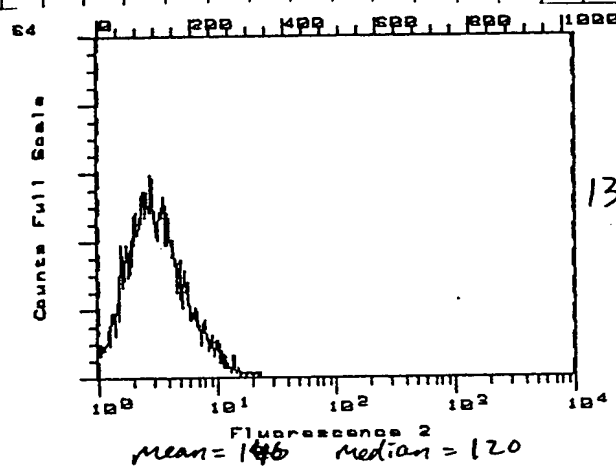
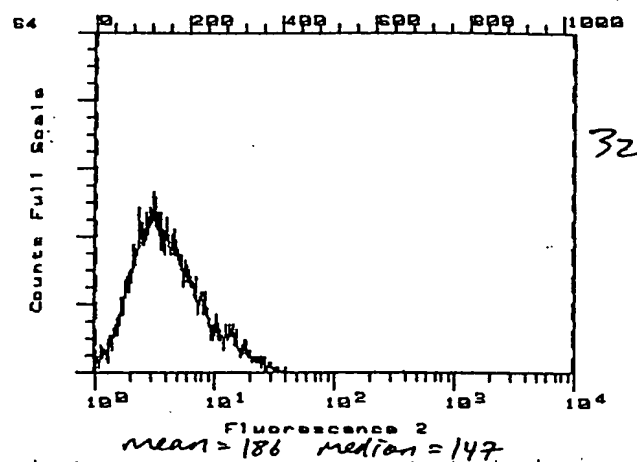
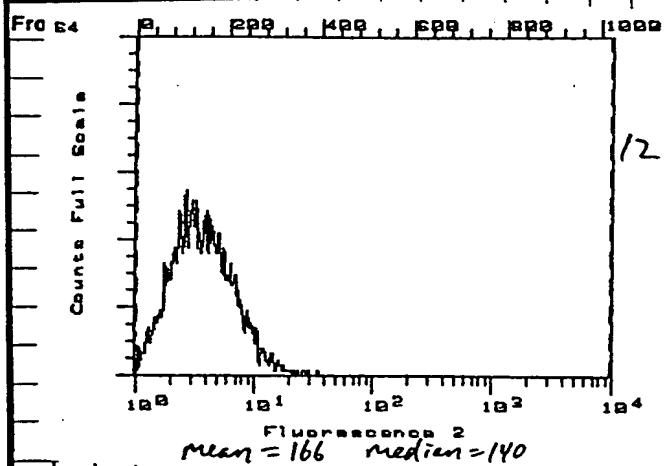
Invented by \_\_\_\_\_

Date THURS

Recorded by WHL

12/2/93

28



29

Witnessed & Understood by me,

Date

Invented by

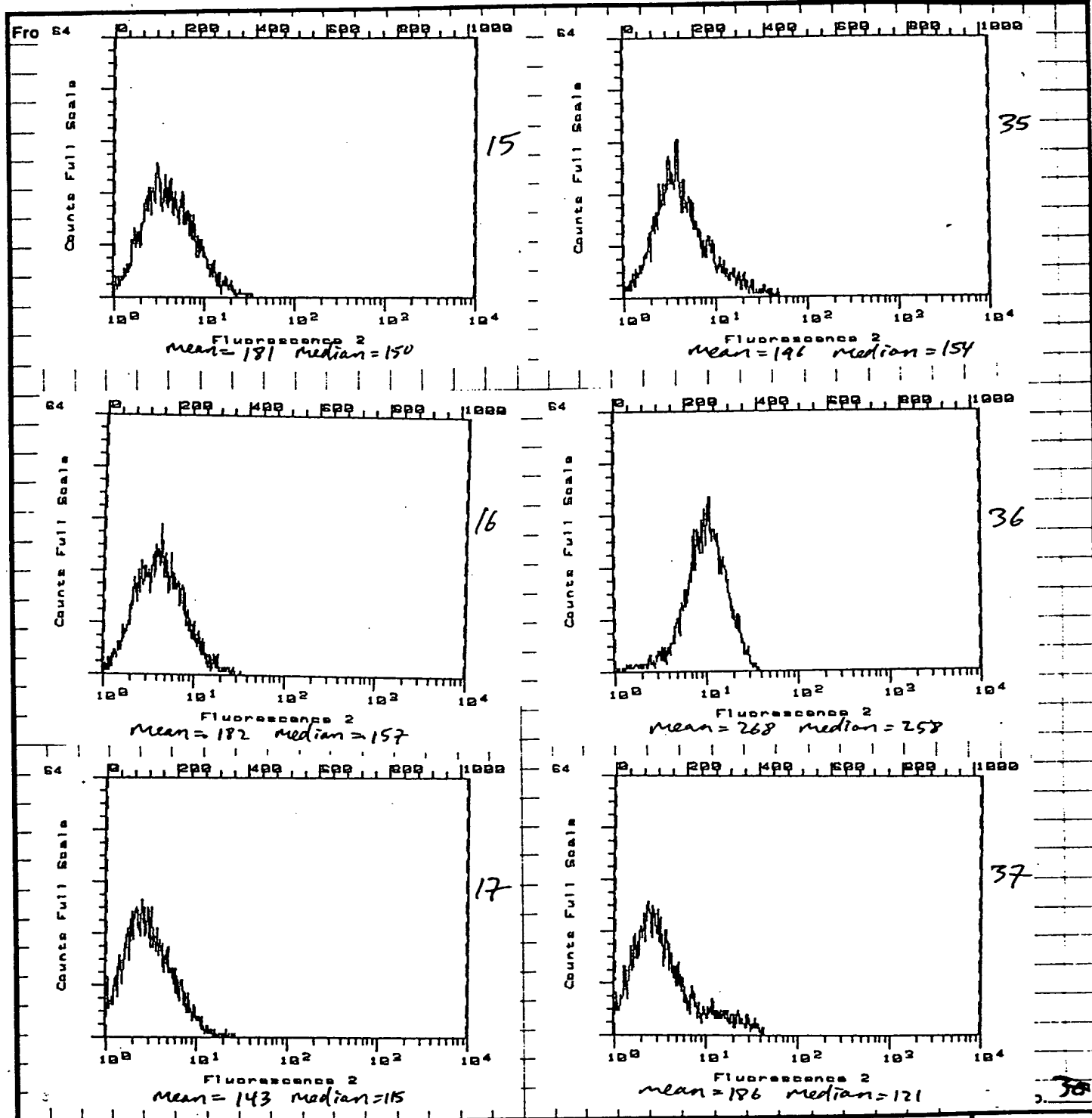
Date

Recorded by

THURS

12/2/93

TITLE \_\_\_\_\_

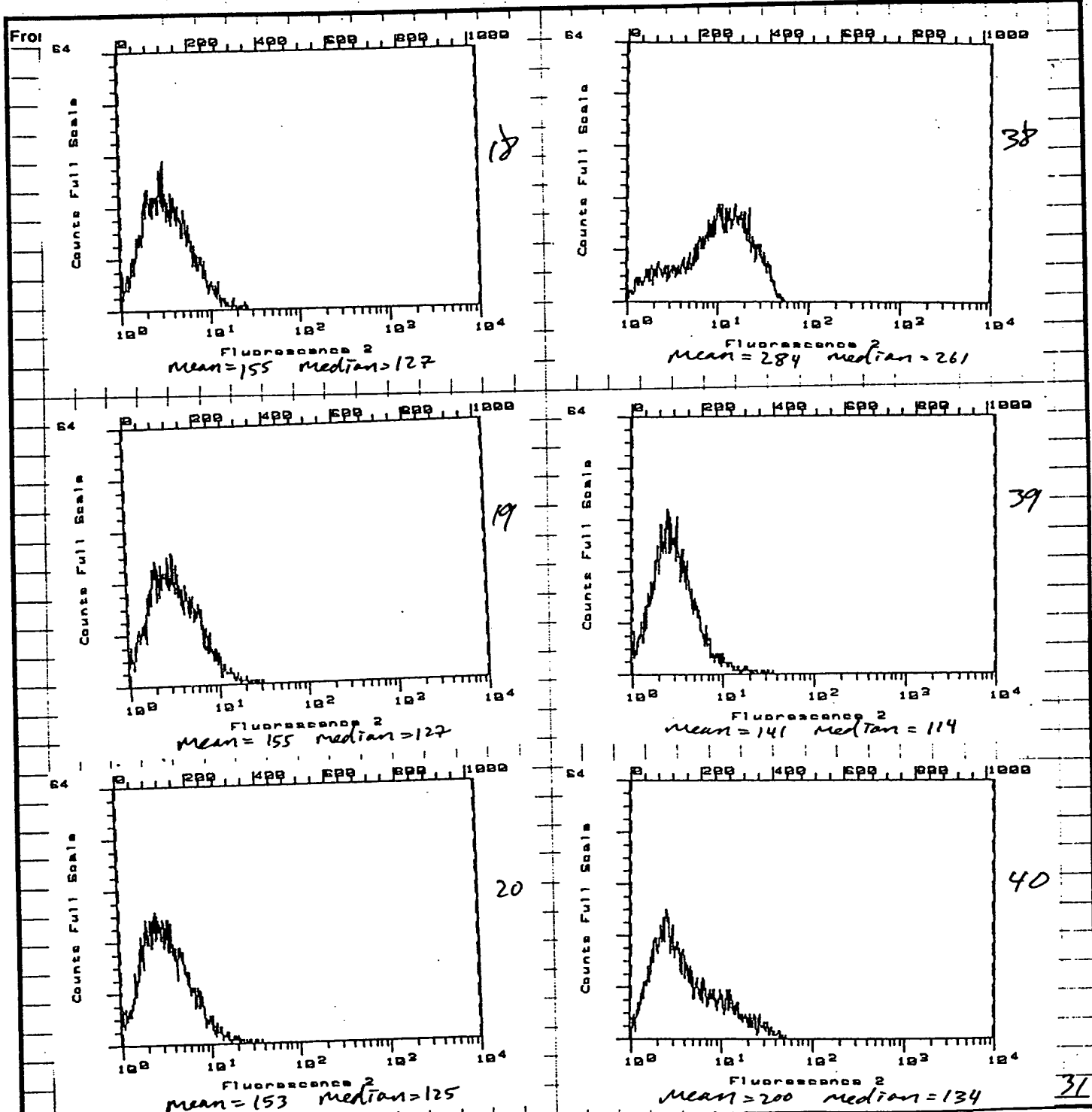


Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	THUE

*W. B. Barton* 12/2/93

Project 1713  
Book No. 19952 TITLE \_\_\_\_\_

30



Witnessed & Understood by me, \_\_\_\_\_

Date \_\_\_\_\_

Invented by \_\_\_\_\_

Recorded by \_\_\_\_\_

Date THURS

12/2/93

Will Brown



||||

Project No. 1713  
Book No. 19952

Exhibit K, pg. 25 of 33

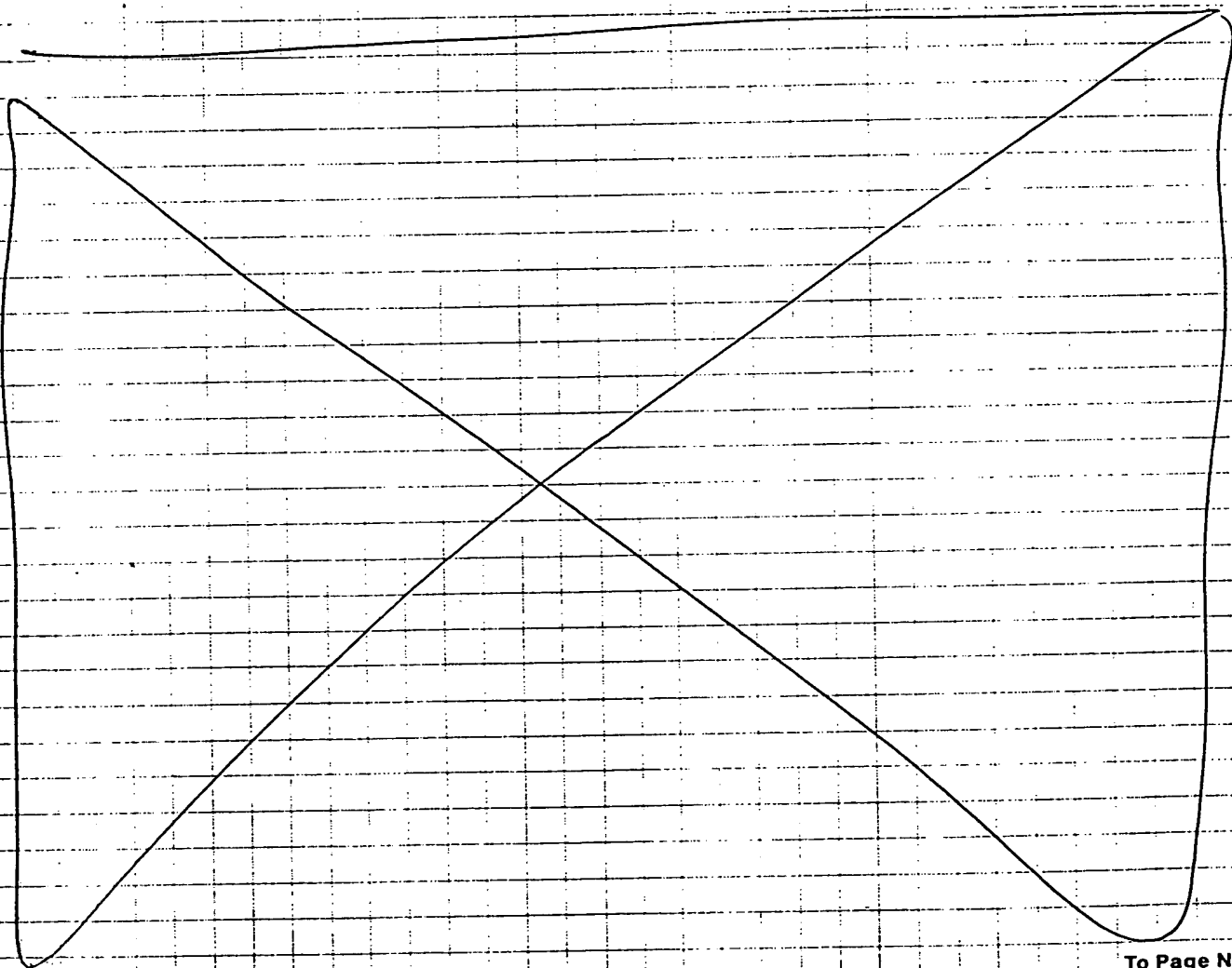
TITLE \_\_\_\_\_

From Page No. 30

Clones 16 & 18 are best expressors.

I will use #16 → probably do a high  
sort soon.

Froze down all other clones & stored -70°C



To Page No. 32

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Date

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Date THURS

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12/2/93

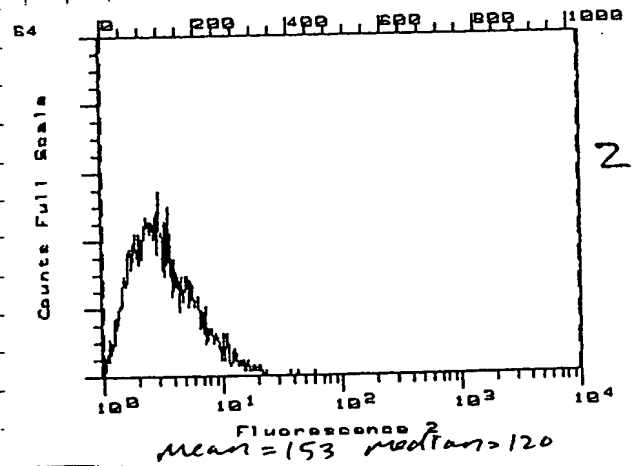
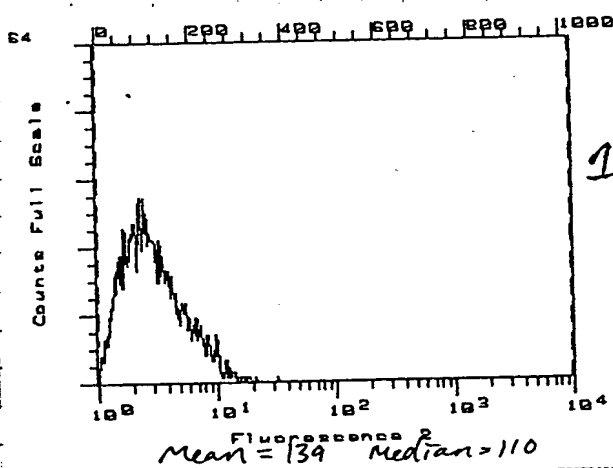
W. F. Bacon

Project No. 1713  
 Book No. 19952 TITLE \_\_\_\_\_

32

From Page No. 31Split spinnersSplit Cto FUS 9 & 11 \* 487 platesDid (repeated) TK6/IsG binding (FACS) on O.K. 4G120

1)	4120	+	1 $\mu$ g/ml	HTTK6
2)	4120	+	5 $\mu$ g/ml	TK6
3)	4120	+	10 $\mu$ g/ml	TK6
4)	4120	+	1 $\mu$ g/ml	CD4
5)	4120	+	2° only	
6)	OK	+	1 $\mu$ g/ml	TK6
7)	OK	+	5 $\mu$ g/ml	TK6
8)	OK	+	10 $\mu$ g/ml	TK6
9)	OK	+	1 $\mu$ g/ml	CD4
10)	OK	+	2° only	

To Page No. 33

Witnessed &amp; Understood by me,

Date

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Recorded by

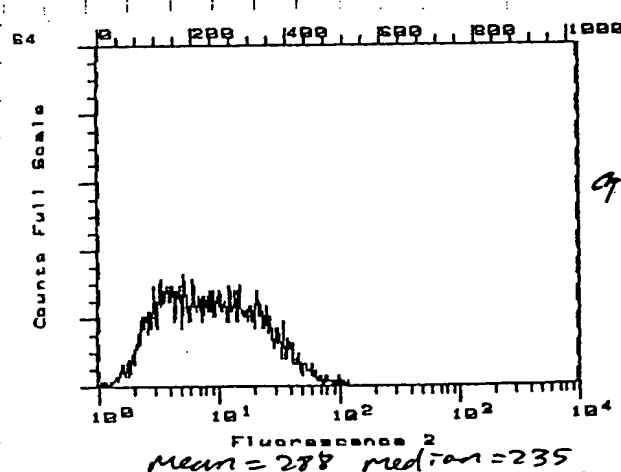
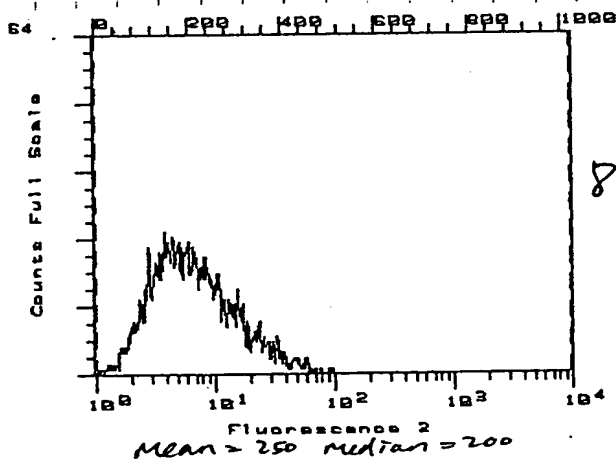
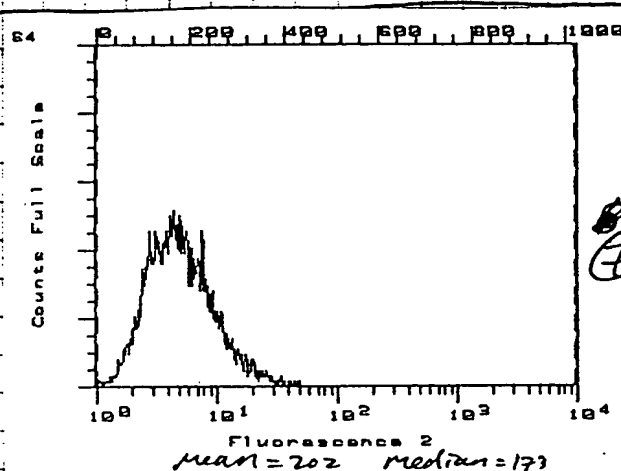
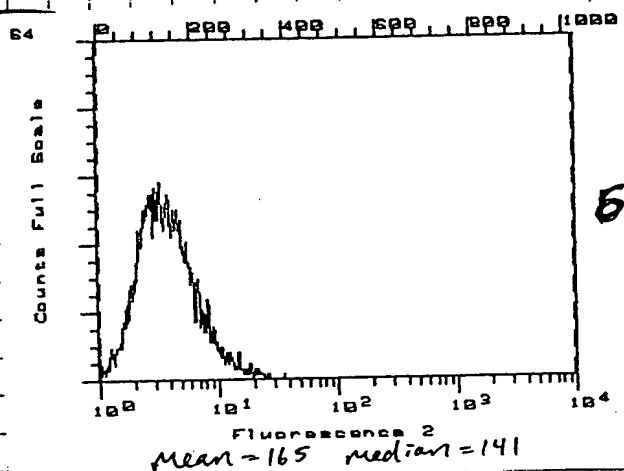
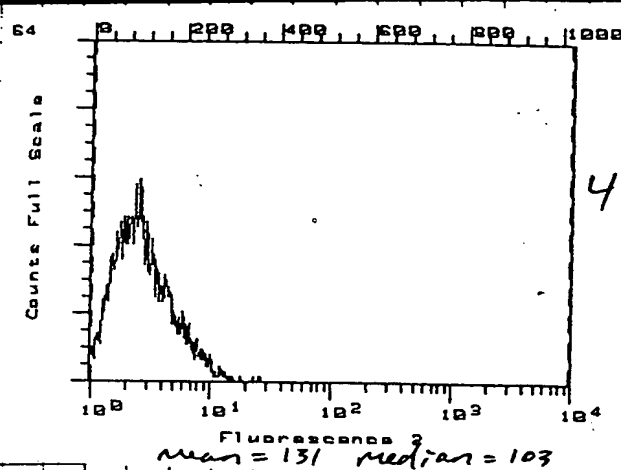
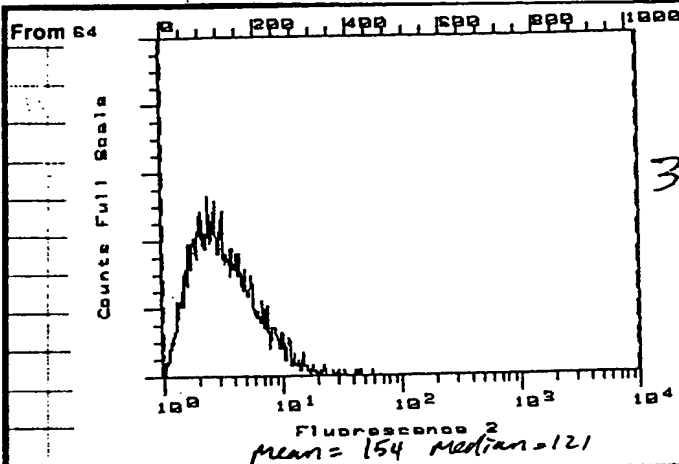
Date FR1

12/3/93

TITLE

Project No. 19952  
Book No. 19952

Exhibit K, pg. 27 of 33



Witnessed & Understood by me,

Date

Invented by

Date FR1

Recorded by WTH/Perown

12/3/93

33

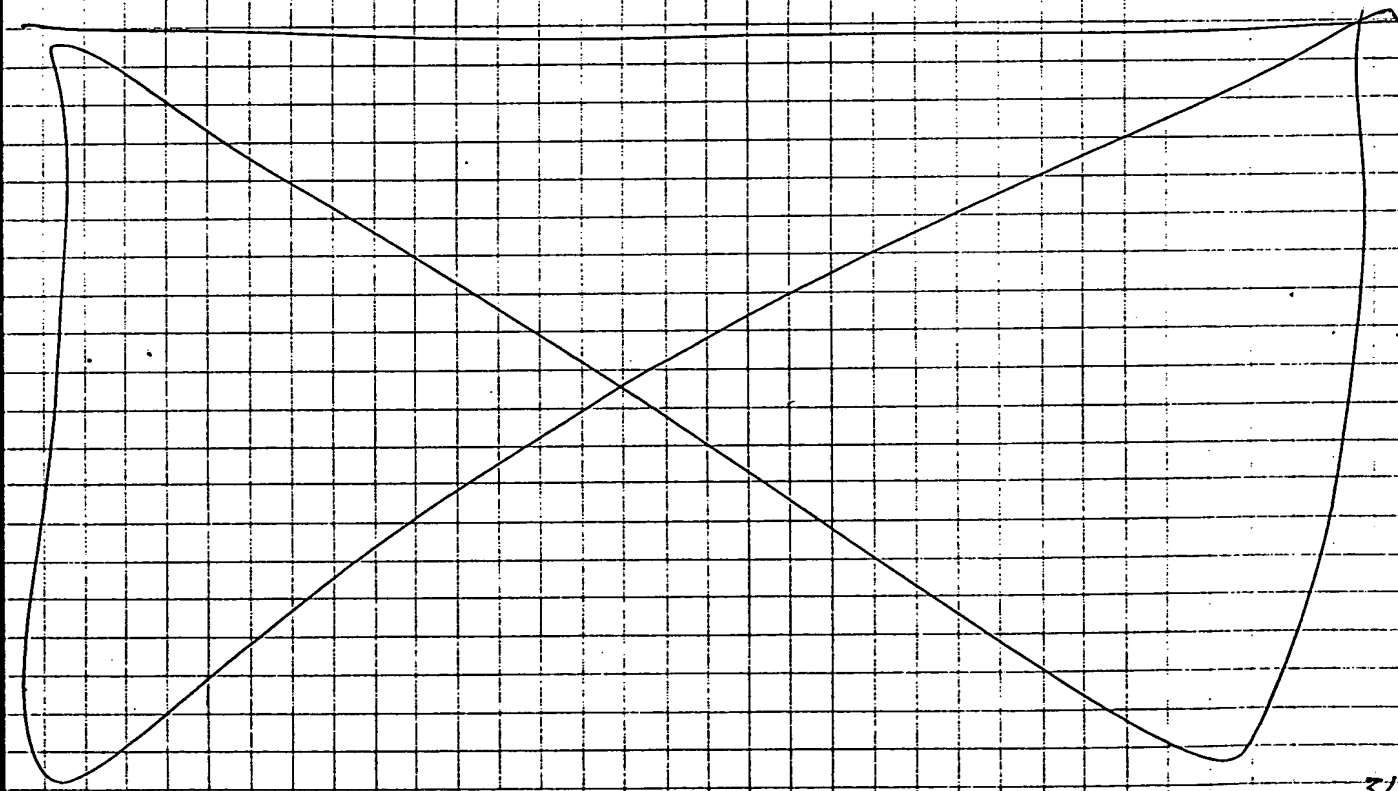
34

From Page No. 33

Histograms were not printed (by error)  
for 2° Ab' controls

It appears that o.k. may be either binding  
Fc or else CD4.

Neither cell line shifts w/ TK6 at  
1 µg/ml which should be noticeable  
if it was going to be physiological.

To Page No. 36

Witnessed &amp; Understood by me,

Date

Invented by

Recorded by

Will BaconDate FR/12/3/93

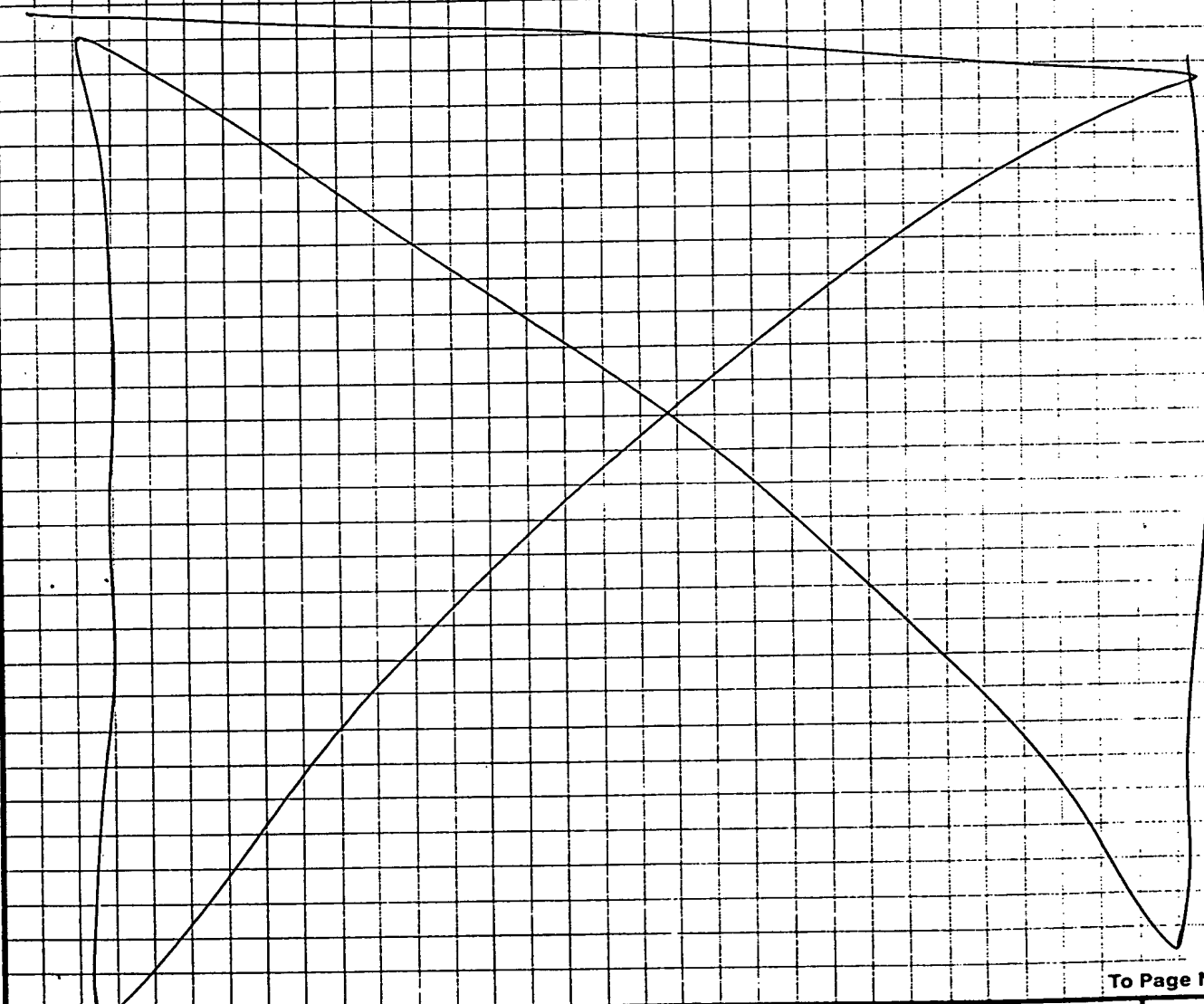
Project No. 1713  
Book No. 19952

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TITLE \_\_\_\_\_

From Page No. 34

Split spinners.  
Checked PSD4 cultures.  
Split O.K. , 6120 & 487



To Page No. 3

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by <u>W. M. Bacon</u>	<u>MON</u> <u>12/6/93</u>

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From Page No. 39Checked all spinners & platesTransferred CHO TK6 Full #16 to WARAK CHAURAPONG  
to screen MoAb fusions by FACS.Got CHO cells from BankingTo Page No. 37

Witnessed &amp; Understood by me,

Date

Invented by

Recorded by

Date TUES12/7/93

TITLE

From Page No. 36

Harvested all P504

Filtered through  $0.22 \mu\text{m}$

Added protease inhibitors (PMSF, Pepstatin, Leupeptin, Aprotinin)

Stored  $4^{\circ}\text{C}$

KPB wants to try IP'ing HRTK6 w/ polyclonal  
& to stimulate.

Plated  $2.5 \times 10^5$  cells each of Full length

#'s 16, 17, 18, 19, 20 & CHO DP-12  
(Others frozen).

into 7 wells of 6 well plates.

Started CHO Full #16 & DP-12 in spinner flasks

To Page No. 38

Witnessed & Understood by me,

Date

Invented by

Recorded by

Date WED

12/8/93

From Page No. 37

Ran prot A on 2 L P504

Washed, eluted, desalted  
Stored 4°C o/n.

Split PMS spinners

Checked all plates

I have an idea that HPTK6 may be a receptor  
for NGF.

Along w/ James Treanor I will try binding

 $^{125}$ I-NGF to CHO Full #16.

Will try in duplicate

 $8 \times 10^6$  cells $4 \times 10^6$  cells $2 \times 10^6$  cells $1 \times 10^6$  cells

Should see an increase in binding w/ more cells

PRE-COUNT

Pat. Group	Tray	Chl Tube	CFM	CFM %CV	CFM	COMMENTS
1	1001	A	117309	0.00	117309	8 A
2	1002	A	120426	0.00	120426	8 B
3	1003	A	119263	0.00	119263	4 A
4	1004	A	119670	0.00	119670	4 B
5	1005	A	120283	0.00	120283	2 A
6	1006	A	119171	0.00	119171	2 B
7	1007	A	119229	0.00	119229	1 A
8	1008	A	118300	0.00	118300	1 B

Page No. 39

Witnessed &amp; Understood by me,

Date

Invented by

Recorded by

Date

JHRS

12/9/93



Proc No. 1713  
Book No. 19952

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TITLE \_\_\_\_\_

From Page No. 32

Dec 09 1993		Report for CFM/3		6:35:33 PM	
RUN ID:12/09/93 18:34		INSTRUMENT ID:9210285		CNT TIME: 1.00	
Pat.   Tray Ch   CFM   CFM			CFM/3	COMMENTS	
Group   Tube			( )		
1A 1	1001 A	809			
	1002 A	1096			
	1003 A	1150			
1B 2	A	1019	17.99	1019	
	1004 A	1642			
	1005 A	1657			
2A 3	1006 A	1337			
	A	1545	11.69	1545	
	1007 A	1596			
2B 4	1008 A	1559			
	1009 A	1747			
	A	1634	6.12	1634	
4A 5	1010 A	1171			
	1011 A	1408			
	1012 A	1629			
4B 6	A	1403	16.33	1403	
	1013 A	1808			
	1014 A	1371			
8A 7	1015 A	1661			
	A	1613	13.77	1613	
	1016 A	1419			
	1017 A	1774			
	1018 A	1180			
	A	1458	20.50	1458	
	1019 A	1443			
	1020 A	1615			

Dec 09 1993		Report for CFM/3		6:38:18 PM	
RUN ID:12/09/93 18:37		INSTRUMENT ID:9210285		CNT TIME: 1.00	
Pat.   Tray Ch   CFM   CFM			CFM/3	COMMENTS	
Group   Tube			( )		
8A	1001 A	1538		8A = 1532	
	1002 A	1309			
	1003 A	1531			
8B	A	1459	8.94	8B = 1398	
	2 1004 A	1355			

No increase in counts with increase in cells.  
Will need to repeat after high sort is  
done on #16.

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Witnessed & Understood by me, \_\_\_\_\_

Date \_\_\_\_\_

Invented by \_\_\_\_\_

Date THURS

Recorded by WJA/Bacon

12/9/93